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博士論文

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（B7およびIFN-γ遺伝子導入腫瘍細胞による抗腫瘍効果の誘導）

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SUMMARY

The antitumor effect of murine tumor cells transfected with murine B7-1 (B7) and murine interferon-gamma (IFN-γ) was examined. An immunogenic MethA sarcoma transfected with B7 induced a tumor-specific antitumor effect *in vivo*, whereas a poorly immunogenic colon26 adenocarcinoma transfected with B7 (B7/colon26), which expressed a low level of MHC class I molecules, could not. Retroviral transfection of IFN-γ into colon26 and B7/colon26 resulted in IFN-γ/colon26 and IFN-γ/B7/colon26, respectively (both of which produced more than 20 units/10^6/24h of IFN-γ). IFN-γ/colon26 and IFN-γ/B7/colon26 upregulated expression of endogenous class I molecules. When 10^6 transfectants were inoculated subcutaneously into mice, IFN-γ/B7/colon26 showed growth retardation of tumorigenicity compared to B7/colon26. In a therapy model, boosting by intraperitoneal injections with IFN-γ/B7/colon26 significantly prevented the growth of subcutaneously inoculated wild type colon26. In addition, IFN-γ/B7/colon26 could induce specific cytolytic activity *in vitro*, using splenocytes from mice immunized by IFN-γ/B7/colon26. These results suggest that transfection of poorly immunogenic tumor cells with both B7 and IFN-γ is more effective than B7 alone in inducing an *in vivo* antitumor effect.

INTRODUCTION

In cancer gene therapy, one of the therapeutic modalities is to use genetically engineered tumor cells as a tumor vaccine (1). In a mouse model, various genes for adhesion molecules and cytokines were transduced into tumor cells, and antitumor effects using these transfected tumor cells have been examined recently (2, 3, 4). Among the adhesion molecules, B7-1 (also known as B7/BB1 or CD80; hereafter we refer to this molecule as B7) is probably the most important, because it is thought to be the most potent co-stimulator in T cell activation (5, 6). When B7 is transduced into mouse melanoma cells, they induce antitumor effect *in vivo* mediated by $CD8^+$ T cells, without requiring $CD4^+$ T cells (2). There have also been reports of many cases of antitumor effects achieved using cytokine gene-transfected tumor cells (7). Interferon-gamma (IFN-γ) is a member of the T helper 1 (Th1) cytokines, which induce cell-mediated immune responses (8, 9), and is currently being tested for transductions into tumor cells as a tumor vaccine (10, 11, 12, 13, 14, 15). The observation that tumor cells transfected with IFN-γ upregulated their major histocompatibility complex (MHC) molecules was considered to be one of the reasons why the transfectants could decrease tumorigenicity (10). In this study, we examined the antitumor effect of genetically engineered tumor cells doubly transfected with B7 and IFN-γ.

MATERIALS AND METHODS

Mice

Female BALB/c A Jcl mice, 6-8-week-old, were purchased from CLEA Japan. Inc., and were maintained in Institute for Experimental Animals, Tohoku University School of Medicine.

Cell lines

The MethA fibrosarcoma line was a methylcholantrene-induced tumor. The BALB/c-derived colorectal carcinoma colon26 line was obtained from Cancer Cell Repository, Institute of Development, Aging, and Cancer, Tohoku University School of Medicine. B16 melanoma was derived from C57BL/6 mice. All of these cell lines were maintained in complete medium, that is, RPMI 1640 (GIBCO BRL, Life Technologies, Tokyo, Japan) supplemented with 10% heat-inactivated fetal calf serum (FCS) (Dainippon Pharmaceutical, Osaka, Japan) , 2 mM L-glutamine (Wako Pure Chemical Industries, Osaka, Japan), 1 mM HEPES (Dojindo Laboratories, Kumamoto, Japan), 100 U/ml penicillin G (Banyu Pharmaceutical Co., Ltd., Japan), 100 μg/ml streptomycin (Meiji Seika Kaisha, Tokyo, Japan), and 2.0 mg/ml sodium bicarbonate (Wako Pure Chemical Industries). All cell lines were cultured at 37℃ in 5% CO_2 and were passed before confluence was achieved using a 1-5-min incubation in 0.53mM EDTA and 0.05% trypsin (GIBCO BRL, Life Technologies) . The packaging cell line ψ CRIP, used for retroviral infection, was maintained in Dulbecco's modified Eagle's medium (DMEM) (GIBCO BRL, Life Technologies) containing 10% FCS.

Transfection of tumor cell lines with plasmid vectors

Murine B7 cDNA was subcloned into the expression vectors BCMGSNeo (obtained from Dr. Karasuyama, Tokyo Metropolitan Institute of Medical Science) and pMKITNeo (a gift from Dr. Maruyama, Tokyo Medical and Dental University), resulting in BCMGSNeo-B7 and pMKITNeo-B7, respectively. MethA lines were transfected with BCMGSNeo-B7 using Lipofectin Reagent (GIBCO BRL, Life Technologies), and they were cultured for 24 hours in complete medium before the addition of 1 mg/ml of the neomycin analog, G418 (Geneticin; Wako Pure Chemical Industries). After selection, limiting dilution was performed. MethA was also transfected with BCMGSNeo in the same manner. The plasmid vector pMKITNeo-B7 was linearized by the restriction

enzyme Sma I (GIBCO BRL, Life Technologies). The colon26 line was transfected with linearized pMKITNeo-B7 by electroporation (Cell Porator; BRL, Life Technologies) at 300 V and 1000 μF. After selection by 0.8 mg/ml Geneticin, each clone was stained by anti-B7 (RM80) mAb with fluorescein-isothiocyanate(FITC)-conjugated anti-rat IgG (PharMingen, San Diego, CA) and was sorted by FACStar cell sorter (Becton Dickinson, San Jose, CA). As a control, pMKITNeo was transfected into colon26 in the same manner.

Transfection of tumor cell lines with retroviral vectors

A retroviral vector MFG (16) and an amphotropic packaging cell line ψ CRIP(17) were used for transfection with murine IFN-γ according to methods described elsewhere (18, 19). Briefly, the retroviral pMFG plasmid was subcloned with the *Nco* I - *Bam* H I fragment of murine IFN-γ cDNA into *Nco* I /*Bam* H I sites and was transfected into ψCRIP (IFN-γ/ψCRIP). These transfected ψCRIPs were maintained in DMEM containing 10% FCS. IFN-γ/ψ CRIP produced MFG/IFN-γ within their supernatant. Colon26 and B7/colon26 were subcultured at a concentration of 1×10^5 cells/25-cm^2 flask. The following day, a 24h-retrovirus supernatant was collected from IFN-γ/ψCRIP culture. Tumor cells in 2 ml of fresh complete medium were incubated with 3.5 ml of MFG/IFN-γ supernatant containing 8 μg/ml of polybrene (Sigma Chemical Co., St. Louise, Mo), at 37℃ for 24h. Following the transfection, the retroviral supernatant were removed and transfected tumor cells were grown in complete medium.

Flow cytometric analysis

The expression of B7 by the cell lines was analyzed by FACScan flow cytometer (Becton Dickinson) using anti-B7 (RM80) mAb with FITC-conjugated anti-rat IgG (PharMingen), and the expression of MHC class I was also analyzed using anti-H-2D^d, anti-H-2K^d, and anti-H-2L^d mAbs (PharMingen) with FITC-conjugated anti-mouse IgG (PharMingen). Data were processed by the Consort 30 program.

IFN-γ assays

For detecting the production of IFN-γ, we performed an enzyme-linked immunosorbant assay after the avidin-biotinylated enzyme peroxidase complex method(20) (ABC-ELISA) according to a method described previously (21). At

first, 96-well plates for ELISA (Immulon 2; Dynatech, Toyama, Japan) was coated with non-neutralizing anti-IFN-γ mAb R4-6A2 at a concentration of 5 μg/ml and incubated overnight at 4℃. After blocking using a skim milk solution (Block Ace; Snow Brand Milk, Sapporo, Japan), supernatants of media in which tumor cells were cultured were loaded in triplicate onto the plate as samples. Recombinant IFN-γ (PharMingen) was also added in duplicate as a standard. The plate was incubated for more than 2 hours at 37 ℃. Then, addition of biotinylated anti-IFN-γ mAb (PharMingen) at a concentration of 8 μg/ml and 1 hour incubation at room temperature were followed by addition of avidine-biotinylated horseradish peroxidase complex (Vectastain ABC kit: Vector Laboratories, Inc., Burlingame, CA). The plate was developed with 1 mg/ml OPD (*o*-phenylenediamine; Wako Pure Chemical Industries) solution containing 0.03% hydrogen peroxide, and stopped with 1 M H_2SO_4. Absorbance at OD490 nm was measured on a microplate reader (2550-UV; Bio-Rad, Hercules, CA).

Tumor establishment model

Tumor cells were harvested and washed twice in phosphate-buffered saline (PBS). They were adjust to the required concentration with 0.1 ml PBS and inoculated into the left flank of BALB/c mice subcutaneously. Tumor growth was measured twice a week using a caliper, and was recorded as the major axis (a) and the minor axis (b). Tumor volume was calculated according to the formula: $V=a\times b^2\times 0.4$ (37). Mice were sacrificed when the tumor size was beyond 20 mm or severe ulceration on the tumor was found. The tumor size at specific dates in each group was analyzed using non-parametric test (Mann-Whitney *U* test), and the survival rates of mice were analyzed using log rank test.

Tumor therapy model

Mice were inoculated with wild-type colon26 at a dose of 5×10^4 cells per mouse (the minimal tumorigenic dose). This was followed by intraperitoneal injection of 5×10^6 tumor cells, including transfectants, treated with mitomycin C (MMC; Kyowa Hakko, Tokyo, Japan), 3 times (day 3, day 10, and day17). Injection of PBS was used as a control. Tumor growth was assessed twice a week by inspection and palpation. After adequate observation of tumor growth, mice were euthanized. The difference of percentage tumor-

free mice was calculated by two-sample Wilcoxon test.

Cytotoxicity assay

Splenocytes were harvested either from naive mice or from mice immunized with B7 transfectant. Then, a mixed lymphocytes-tumor culture (MLTC), i.e. coculturing of splenocytes with MMC-treated colon26 or B7 transfectant, was performed. Five days after MLTC, the effector cells were harvested, serially diluted, and incubated with 10^4 ^{51}Cr-labeled target cells in 96-well, round bottom plates (Becton Dickinson). Target cells (1×10^6) were labeled with 100μCi of ^{51}Cr sodium salt for 1 h. After 4-h incubation at 37℃, supernatants from each well were harvested and surveyed by γ-scintillator. The percent specific lysis was calculated according to the formula: (experimental cpm - spontaneous cpm)/(maximal cpm - spontaneous cpm)$\times$ 100. For a comparison of percent cytotoxicity of effector cells at the same E/T ratio, we analyzed the data by Student's t-test.

RESULTS

In vivo antitumor effect of B7-transfected tumor cells

Transfection by Lipofectin with BCMGSNeo-B7 into MethA resulted in B7/MethA. High expression of B7 was seen in B7/MethA compared to MethA, which had no expression of B7 (Fig.1A). On the other hand, weak expression of B7 was originally observed in colon26 (Fig.1B). To get a high level of B7-expression, transduction of B7 cDNA into colon26 was performed. Since we could not get B7 transfectants by the Lipofectin method in the case of colon26, we tried electroporation, which was successful. After selection and sorting, highly expressing B7 clones (B7/colon26) were obtained (Fig.1B). The high level of B7 expression was preserved after more than 20 passages. B7 expression by tumor cells transfected with backbone plasmids, i.e. neo/MethA and neo/colon26, were the same as MethA and colon26, respectively (data not shown). Growth rates of the transfectants *in vitro* were also the same as those of wild types (data not shown).

To examine the antitumor effect of B7 transfectants, 1×10^6 viable tumor cells were inoculated into the left flank of mice subcutaneously. All of the mice injected with MethA allowed tumor to grow and were sacrificed within 2 weeks due to severe ulcerations. Although B7/MethA seemed to grow in a few days after inoculation, all of them began to regress soon and were rejected after all (Fig.1C). These mice which rejected B7/MethA were rechallenged by 10^6 of wild type MethA (in the left flank) and the same dose of wild type colon26 (in the right flank). No tumor formation was seen on the MethA side, whereas colon26 grew progressively to kill mice (data not shown). The same experiment was performed using colon26 and its B7 transfectant. Colon26, which grew more slowly than MethA, killed all of the mice inoculated. B7/colon26 performed in the same manner as B7/MethA for 3 weeks after inoculation, but then, some of them began to grow again. In the end, half of the mice injected with B7/colon26 were dead due to tumor growth (Fig.1D). Some of the growing B7/colon26 tumors were resected before the death of mice, digested with trypsin, and stained by anti-B7 mAb. FACScan analysis showed that the growing B7/colon26 had the same level of B7 expression as prior to injection (data not shown). Both neo/MethA and neo/colon26 performed like wild types and soon killed mice (data not shown).

Transfection of tumor cells with IFN-γ with or without B7

Since FACScan analysis revealed that colon26 expressed MHC class I more weakly than MethA (Fig. 2A), murine IFN-γ cDNA was transfected into colon26 and B7/colon26 by retroviral infection to upregulate class I molecules. As a result, IFN-γ/colon26 and IFN-γ/B7/colon26 were made. Both IFN-γ/colon26 and IFN-γ/B7/colon26 produced more than 20 units/10^6/24h (15 units/10^6/24h after MMC treatment) of IFN-γ, and expressed MHC class I stronger than colon26 (Fig. 2C) and B7/colon26 (Fig. 2D). IFN-γ transfection did not affect B7 expression (Fig. 2B). Doubling time of IFN-γ/colon26 *in vitro* was the same as colon26, whereas IFN-γ/B7/colon26 grew relatively slowly (data not shown).

In vivo antitumor effect of tumor cells transfected with B7 and IFN-γ

We performed a tumor establishment study using the transfectants. Live tumor cells were inoculated into mice subcutaneously at a dose of 10^6 cells per mouse (5 mice/group). Compared to the growth rate of colon26, significant growth retardation was seen in all transfectants, and IFN-γ/B7/colon26 tended to grow slower than B7/colon26 (Fig. 3). But these transfectants could not affect the survival rates of tumor-injected mice (data not shown).

Furthermore, all of the transfectants were examined as a tumor vaccine in a tumor therapy model. Mice were inoculated subcutaneously with 5×10^4 colon26. These mice were intraperitoneally injected with 5×10^6 MMC-treated transfectants on day 3, day 10, and day17, and appearance of the tumor was monitored. All of the mice treated with PBS, colon26 or B7/colon26 exhibited tumor growth within17 days. But, some of the mice treated with IFN-γ/colon26 (17%) or IFN-γ/B7/colon26 (38%) were tumor free. In particular, the IFN-γ/B7/colon26 treatment group significantly prevented tumor growth compared to colon26 and B7/colon26 (Fig. 4). Both in the tumor establishment model and in the tumor therapy model, neo/colon26 yielded almost the same result as wild type colon26 (data not shown).

In vitro demonstration of antitumor effect

To demonstrate an antitumor effect *in vitro*, splenocytes from the mice immunized with IFN-γ/B7/colon26 were cocultured with MMC-treated colon26 or IFN-γ/B7/colon26 for 5 days (MLTC). Cytotoxicity of these effector splenocytes was tested against ^{51}Cr-labeled colon26, MethA, or B16

melanoma target cells. Against colon26 target cells, high level of cytotoxicity was attained by effector cells from MLTC with IFN-γ/B7/colon26, significantly higher than that with colon26 (Fig. 5A). On the other hand, neither of the effectors could kill MethA (data not shown) or B16 melanoma (Fig. 5B). When the splenocytes were harvested from naive mice as effectors, no cytotoxicity was observed against any target tumor cells (data not shown).

DISCUSSION

At first, we examined the *in vivo* antitumor effects of B7-transfected tumor cells. B7, formerly reported as an Ig superfamily activating B cells (22), is expressed together with MHC molecules on antigen presenting cells (APC). It provides the co-stimulatory signal (or the second signal) necessary for T cell activation through CD28 (23). If tumor cells sufficiently express MHC class I (together with so-called tumor-associated antigen), tumor cells transfected with B7 can behave as APCs (24). In this point of view, the well-studied tumor MethA was suitable for B7 transfection. The antigen peptide of MethA recognized by $CD8^+$ cytotoxic T lymphocyte (CTL) has recently been identified (25). In our study, B7-transfected MethA (B7/MethA) was rejected by syngeneic mice after subcutaneous injection, whereas wild type MethA was not. The mice which had rejected B7/MethA prevented tumor growth of rechallenged B7-negative MethA, but failed to block the growth of other syngeneic tumor cell lines. These observations showed that B7-transfected tumor cells could induce a tumor-specific antitumor effect, which is compatible with previous reports (2, 26).

We applied the B7 transfection to colon26, which was reported to be a poorly immunogenic tumor line (27). Surprisingly, wild type colon26 did express B7 weakly. It has been suggested that oncogenic insults could induce host cells to express B7 (28). There have been several reports of B7-expressing melanomas (29), which indicates that the B7 molecule can be expressed by some tumor cells. When we injected mice with B7-transfected colon26 (B7/colon26), which expressed high level of B7, we could not get the same result as with B7/MethA. We hypothesized this difference was due to the poor immunogenicity of colon26, because tumor immunogenicity is said to be critical to the outcome of costimulation of T cell-mediated tumor immunity by B7 (30). Actually, the expression level of MHC class I on colon26 ($H\text{-}2^b$) was very low. Some cytokines were reported to be tranfected into tumor cells together with B7, i.e. IL-2 with B7 (31) or IL-7 with B7 (32), and these combination could induce stronger T cell activation and tumor immunity than when using B7 alone. Among the many cytokines, we paid attention in particular to IFN-γ. Transfection with an IFN-γ gene into tumor cells has been reported to upregulate not only the expression level of MHC molecules but also immunogenicity (10, 33). We additionally made single (IFN-γ/colon26)

and double (IFN-γ/B7/colon26) transfectants, and examined whether they could induce antitumor effects which B7/colon26 could not.

In tumor establishment models, *in vivo* growth rate of IFN-γ/B7/colon26 was much slower than B7/colon26 or IFN-γ/colon26, although not significant. This suggests that double transfectants with IFN-γ and B7 can work more effectively than B7 transfectant in reducing tumorigenesis. In the tumor therapy models, the mice inoculated subcutaneously with wild type colon26 (on day 0) were injected intraperitoneally with MMC-treated transfectants three times (on days 3, 10, 17). We chose the peritoneal cavity as a boosting route because this cavity is known to be rich in immunocytes, and peritoneal exudate cells (PEC) of tumor-inoculated mice are thought to contain antitumor effector cells (27, 34). We also chose MMC treatment, rather than irradiation, for inactivation of boosting trasfectants because irradiation might abrogate the effect of B7 (26). In this experiment, boosting by IFN-γ/B7/colon26 resulted in significant prevention of formerly inoculated tumor growth, compared with boosting by colon26 and B7/colon26. Identification of the effector cells mediating this antitumor effect was not pursued in this study. In former studies using B7, it was reported that $CD8^+$ T cells were required for the protective effect induced by B7 transfected tumor cells and $CD4^+$ T cells were not required (2, 26). IFN-γ is known to upregulate MHC molecules on various cells (35), and indeed IFN-γ transfectants could upregulate their class I molecules. IFN-γ/B7/colon26, which expressed MHC class I higher than B7/colon26, might have induced $CD8^+$ CTL directly. On the other hand, some IFN-γ-transfected cells are reported to activate NK cells as effectors (11, 36). So we suggest that not only specific CTLs but also nonspecific effectors such as NK cells could have mediated the antitumor effect induced by IFN-γ/B7/colon26.

For the *in vitro* study, we harvested splenocytes from naive mice and performed MLTC with wild type colon26 and its transfectants, but failed to induce specific killer cells against colon26 (even MLTC with IFN-γ/B7/colon26 failed). But when we used splenocytes from the mice immunized with IFN-γ/B7/colon26 and performed MLTC with the same transfectant, a high percentage of specific lysis against colon26 was obtained by ^{51}Cr release assay. The same result has been reported using B7 and IL-2 double transfectants (31). Genetically engineered tumor cells are suggested to have the potential to activate previously primed cells *in vitro*, though it seemed

difficult to induce CTLs from naive mice.

In the present study, we showed the usefulness of tumor cells doubly transfected with B7 and IFN-γ as a tumor vaccine. When active immunotherapy with genetically engineered tumor cells is applied clinically, the combination of B7 and IFN-γ should be considered for gene transfection.

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FIGURE LEGEND

Fig. 1 B7 expression and *in vivo* tumor growth of B7/MethA and B7/colon26. MethA and B7/MethA (A) or colon26 and B7/colon26 (B) were stained by anti-B7 (RM80) mAb followed by FITC-conjugated anti-rat IgG secondary antibody. B7 expression was analyzed by FACScan. MethA and B7/MethA (10^6 cells/mouse) were inoculated subcutaneously into the left flank of BALB/c mice (n=6), and tumor growth was monitored (C). The same establishment model was performed using colon26 and B7/colon26 (D).

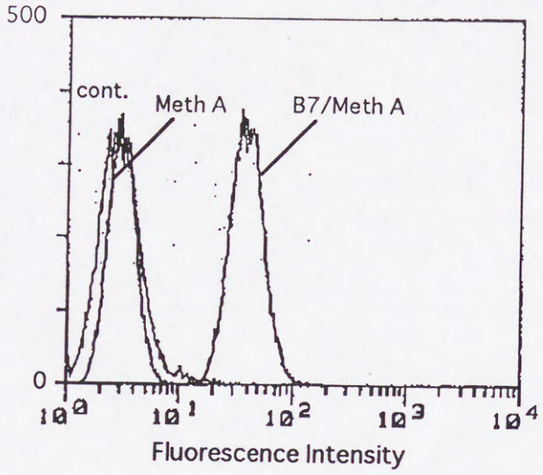
Fig. 2 Upregulation of MHC class I in both IFN-γ/colon26 and IFN-γ/B7/colon26. Wild type colon26 and its transfectants were stained by anti-H-$2D^d$, anti-H-$2K^d$, anti-H-$2L^d$, or anti-B7 mAbs followed by FITC-conjugated anti-mouse IgG secondary antibody, and analyzed by FACScan. The expression of MHC class I was represented by H-$2K^d$, because the same results were obtained in expressions of H-$2D^d$ and H-$2L^d$ in all experiments. A. Class I expression of MethA and colon26. B. B7 expression of colon26 and its transfectants. C and D. Class I expression of colon26 and its transfectants.

Fig. 3 Effect of IFN-γ/B7/colon26 in tumor establishment models. 10^6 of tumor cells in each group were inoculated subcutaneously into mice (n=5 in each group), and tumor growth was monitored. Tumor volume of each group was shown in mean$\pm$SD.

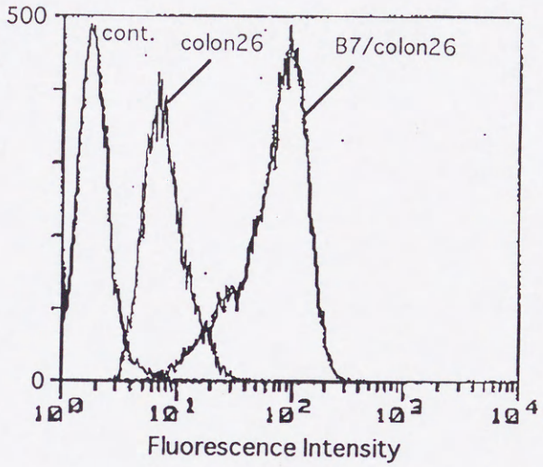
Fig. 4 Effect of IFN-γ/B7/colon26 in tumor therapy models. 5×10^4 of colon26 was subcutaneously injected into mice, which treated by boosting i.p. injection (⬇) for three times with MMC-inactivated wild type colon26, one of the transfectants, or PBS (n=6 in each group). *, $p < 0.05$ versus colon26 and B7/colon26 by two-sample Wilcoxon test.

Fig. 5 *In vitro* induction of cytolytic activity. Splenocytes were harvested from mice immunized with IFN-γ/B7/colon26, and cocultured with MMC-treated colon26 or IFN-γ/B7/colon26 for 5 days. Cytolytic function of these effector cells were tested by 4h ^{51}Cr-release assay against colon26 (A) or B16 melanoma (B) as target cells. Percent cytotoxicity of effector cells induced by MLTC using IFN-γ/B7/colon26 was compared with MLTC using colon26 at the

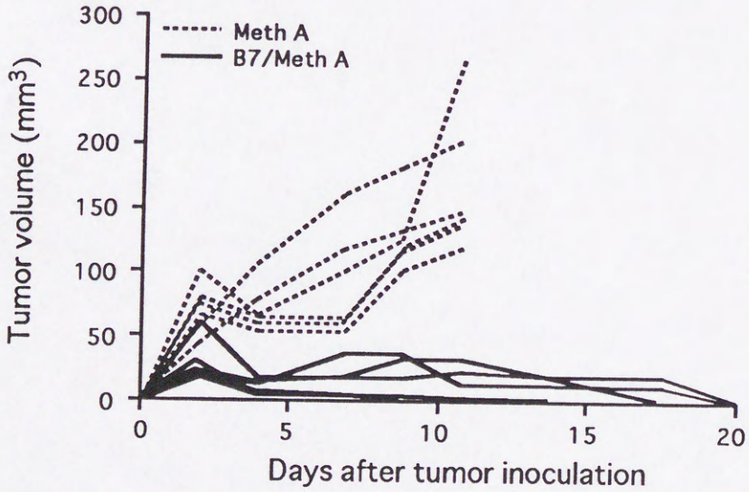
same E/T ratio by Student's t-test (*, $p < 0.05$, **, $p < 0.01$).


500
cont.
Meth A
B7/Meth A
0
10⁰ 10¹ 10² 10³ 10⁴
Fluorescence Intensity

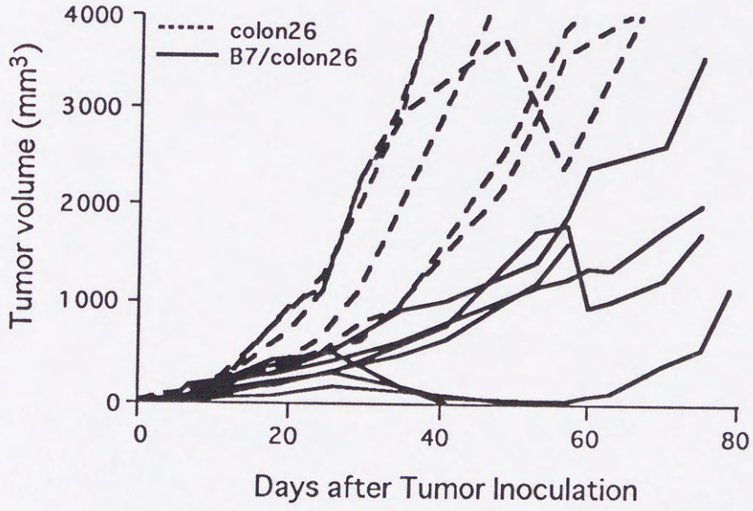
(A)


500
cont.
colon26
B7/colon26
0
10⁰ 10¹ 10² 10³ 10⁴
Fluorescence Intensity

(B)


300
250
200
150
100
50
0
Meth A
B7/Meth A
Tumor volume (mm³)
0 5 10 15 20
Days after tumor inoculation

(C)


4000
3000
2000
1000
0
colon26
B7/colon26
Tumor volume (mm³)
0 20 40 60 80
Days after Tumor Inoculation

(D)

Fig. 1

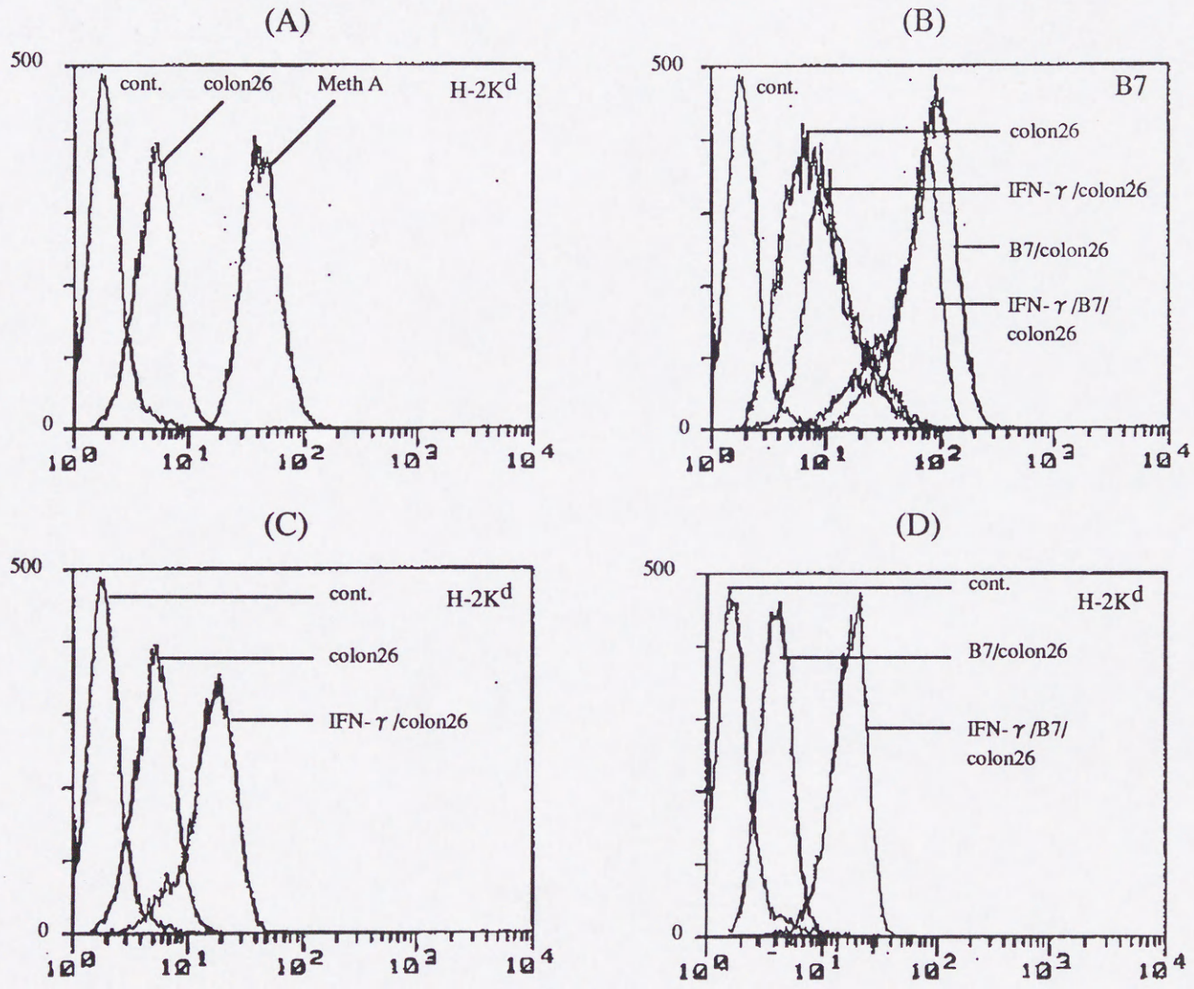

(A)
500
cont.
colon26
Meth A
H-2K^d
0
10^0 10^1 10^2 10^3 10^4
(B)
500
cont.
B7
colon26
IFN-γ /colon26
B7/colon26
IFN-γ /B7/ colon26
0
10^0 10^1 10^2 10^3 10^4
(C)
500
cont.
H-2K^d
colon26
IFN-γ /colon26
0
10^0 10^1 10^2 10^3 10^4
(D)
500
cont.
H-2K^d
B7/colon26
IFN-γ /B7/ colon26
0
10^0 10^1 10^2 10^3 10^4
Fluorescence Intensity

Fig. 2

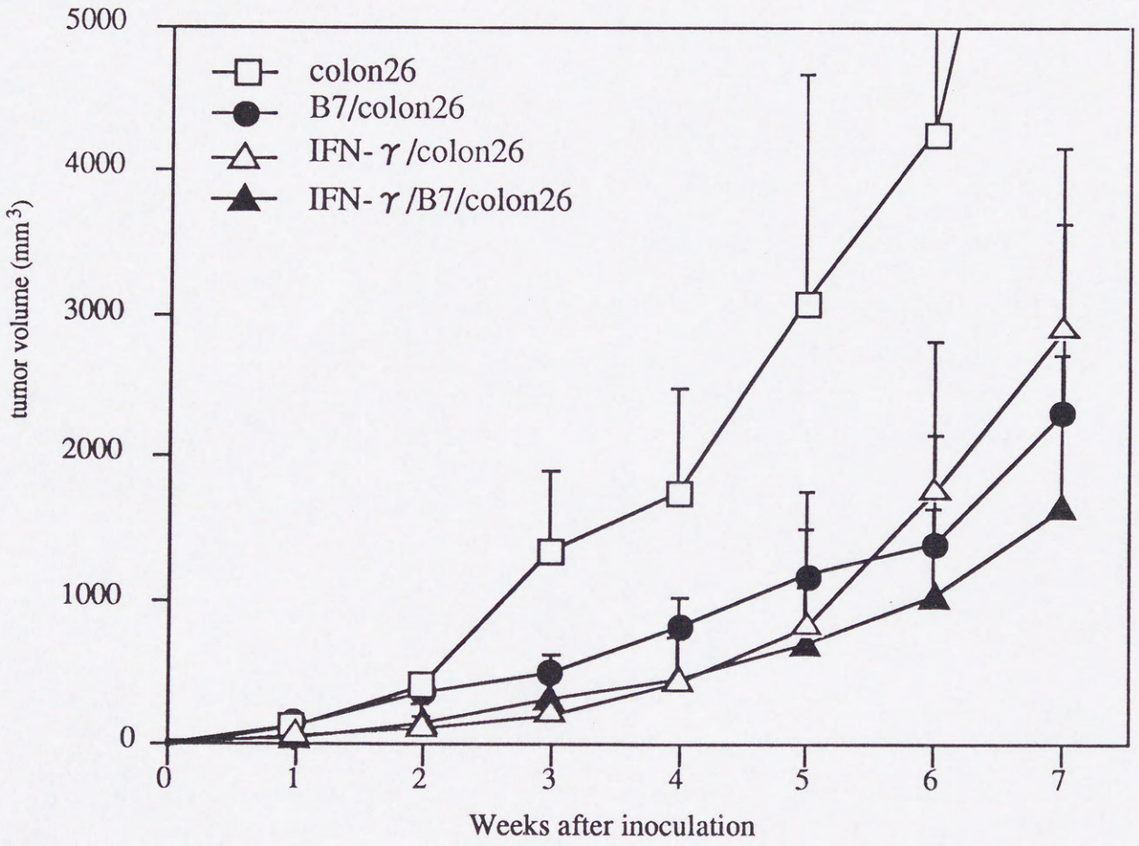

5000
4000
3000
2000
1000
0
tumor volume (mm3)
colon26
B7/colon26
IFN-γ/colon26
IFN-γ/B7/colon26
0
1
2
3
4
5
6
7
Weeks after inoculation

Fig. 3

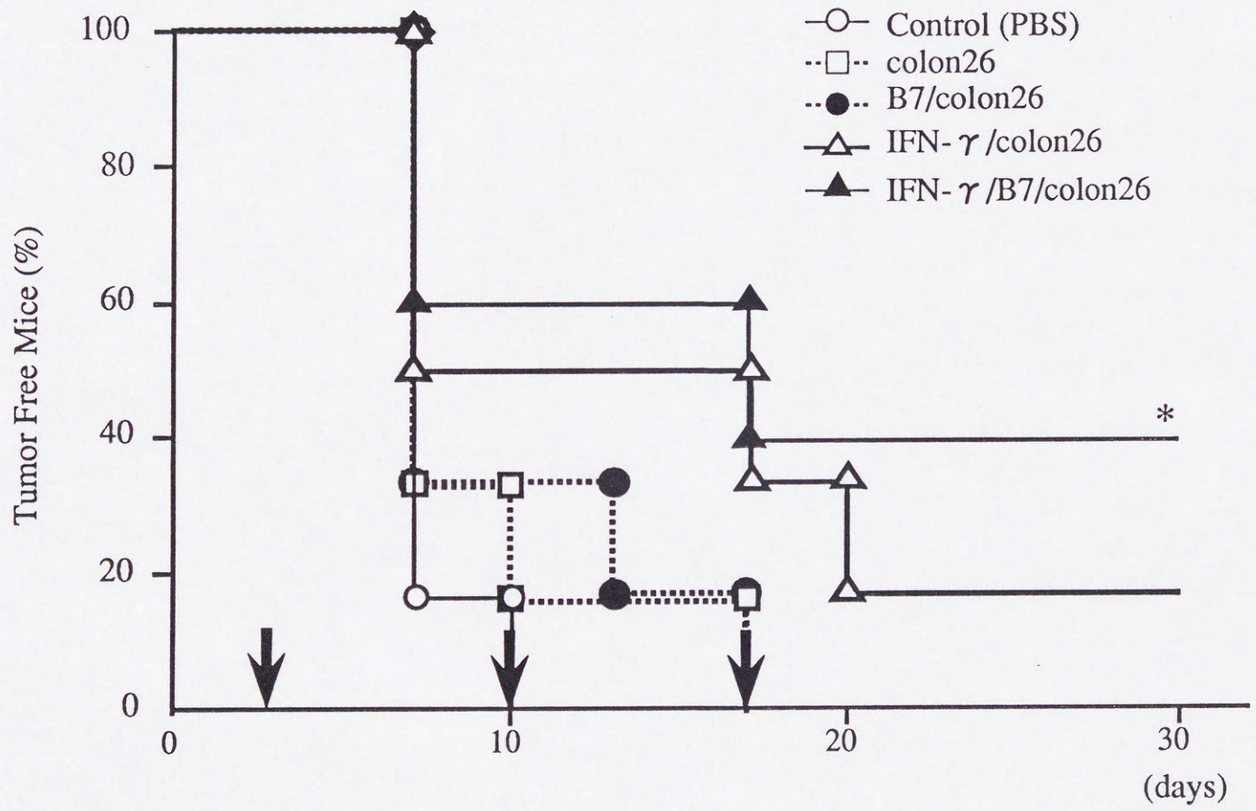

Control (PBS)
colon26
B7/colon26
IFN-γ/colon26
IFN-γ/B7/colon26
Tumor Free Mice (%)
100
80
60
40
20
0
0
10
20
30
(days)
*

Fig. 4

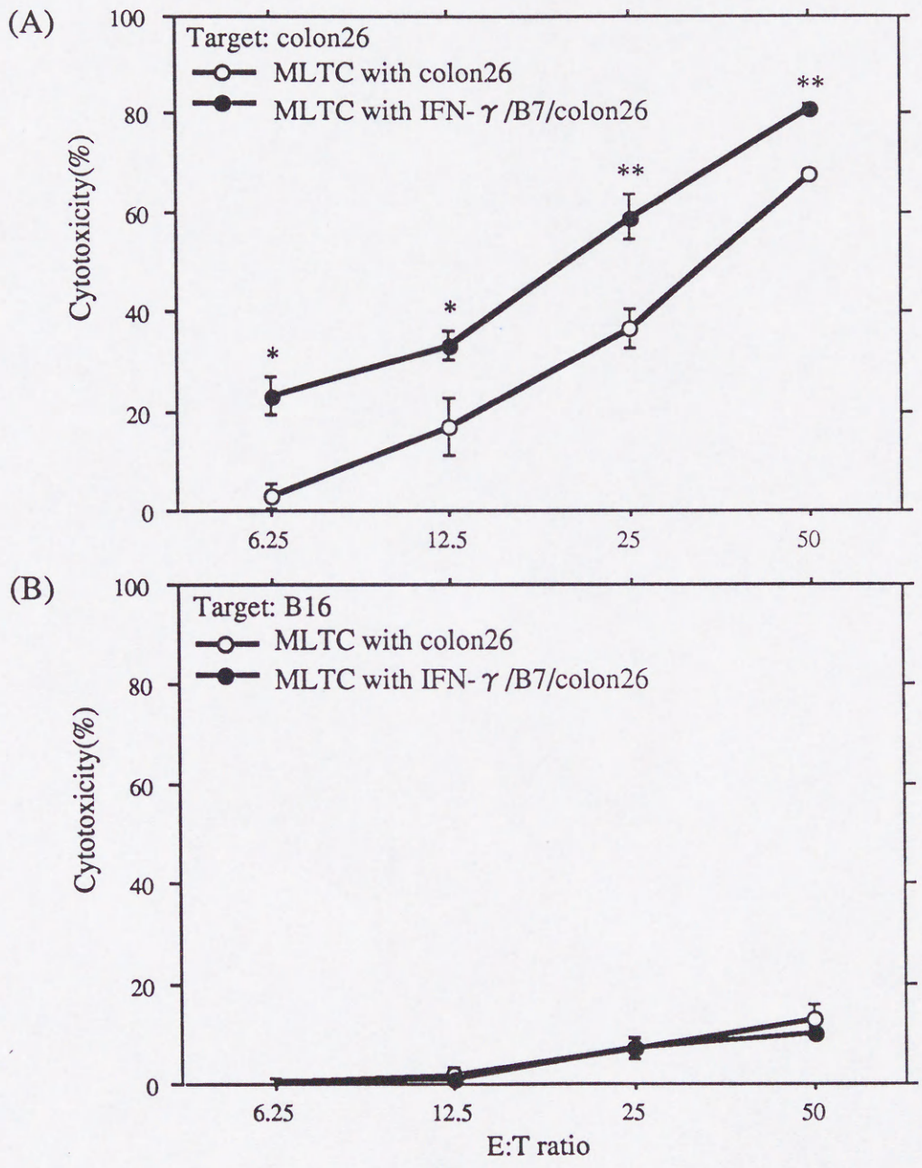
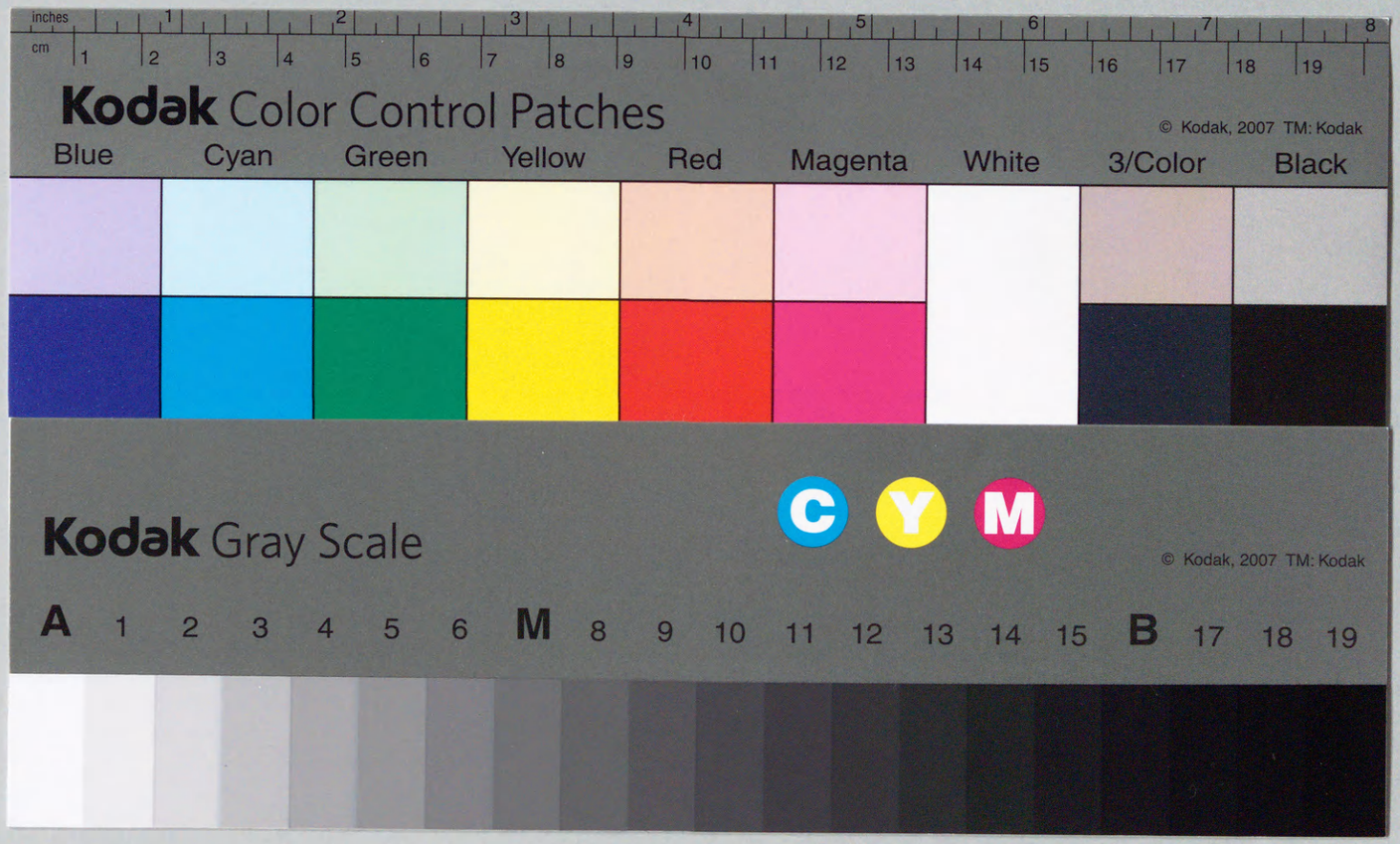

(A)
Target: colon26
MLTC with colon26
MLTC with IFN-γ /B7/colon26
Cytotoxicity(%)
100
80
60
40
20
0
*
*
**
**
6.25
12.5
25
50
(B)
Target: B16
MLTC with colon26
MLTC with IFN-γ /B7/colon26
Cytotoxicity(%)
100
80
60
40
20
0
6.25
12.5
25
50
E:T ratio

Fig. 5


inches
cm
Kodak Color Control Patches
© Kodak, 2007 TM: Kodak
Blue
Cyan
Green
Yellow
Red
Magenta
White
3/Color
Black
Kodak Gray Scale
C
Y
M
© Kodak, 2007 TM: Kodak
A 1 2 3 4 5 6 M 8 9 10 11 12 13 14 15 B 17 18 19